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ASSESSMENT OF AQUATIC IMPACTS IN A AREA TREATED WITH AMINOCARB AT 180 GRAMS PER HECTARE

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INLAND WATERS DIRECTORATE
ATLANTIC REGION
WATER QUALITY BRANCH
MONCTON, NEW BRUNSWICK

ASSESSMENT OF AQUATIC IMPACTS IN AN AREA TREATED WITH AMINOCARB AT 180 GRAMS PER HECTARE

Technical Report

IWD-AR-WQB-85-94

by

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ABSTRACT

This study was designed to measure aquatic residue concentrations and biological impact of high dosage rate (180 g ai/ha) Matacil 180 F sprays which were used in an attempt to control spruce budmoth populations in white spruce plantations. The spray was applied directly to approximately 300 metres of the treatment stream in a single swath. Residue analyses were conducted on water, sediment and freshwater clam (Anadonta sp.) samples obtained at various times after spray. Aminocarb concentrations were below detection limits in all samples with the exception of one water sample taken 4 days after spraying and shortly after a rainfall event.

The total number of aquatic invertebrates in the drift of the treated stream did not change appreciably after the spray. There was also no decrease in the total number of benthic invertebrates colonizing artificial substrates after spray.

Brain acetylcholinesterase activity of brook trout (Salvelinus fontinalis) in the treated stream was not depressed, compared with pre-spray and control fish, immediately after spraying. A number of fish which were found dead in the holding cages at 72 and 96 hours after spray had depressed brain acetylcholinesterase activities, however, this was not believed to be conclusive evidence of pesticide induced mortality.

The results obtained indicate that there was little, if any impact on the treatment stream from the pesticide application. According to our calculations, the principal reason for this is the rapid dilution and short residence time of any aminocarb which reached the stream surface.

RÉSUMÉ

Cette étude a pour but de mesurer les concentrations aquatiques résiduelles et l'impact biologique de doses élevées (180 grammes d'ingrédients actifs/hectare) de Matacil 180F pulvérisé sur des plantations d'épinettes blanches afin de contrôler les populations de tordeuses de l'épinette. L'insecticide fut pulvérisé d'un seul jet sur le cour d'eau dans un corridor de 300 mètres. Des analyses furent effectuées sur des échantillons d'eau, de sédiments et de coques d'eau douce (Anadonta sp.) prélévés à différents intervalles après l'application de l'insecticide. Les concentrations en aminocarb furent inférieures à la limite de détection pour tous les échantillons à l'exception d'un seul échantillon d'eau pris 4 jours après l'arrosage et peu après une averse de pluie.

Le nombre total d'organismes invertébrés aquatiques en aval du cours d'eau n'a pas changé de façon significative après l'arrosage. Il n'y a pas eu non plus de diminution du nombre d'invertébrés dans la faune benthique colonisant des capteurs artificiels.

Comparativement aux poissons étudiés avant l'arrosage et ceux du groupe témoin, l'activité de l'acétylcholinesterase, un enzyme du cerveau, a resté la même juste après l'arrosage chez les truites de ruisseaux (Salvelinus fontalis) habitant le cours d'eau traité. Quoiqu'un certain nombre des poissons gardés en cage et trouvés morts à 72 heures et 96 heures après l'arrosage avaient leur activité en acétylcholinesterase réduite, il n'est pas possible de conclure pour autant que l'insecticide soit la cause des mortalités.

Les résultats obtenus démontrent que l'application de l'insecticide n'a eu que peu d'influence, sinon aucune, sur le cours d'eau traité. Selon nos calculs, la raison majeure de ce fait est la dilution rapide ainsi que le court temps d'exposition de n'importe quel aminocarb qui atteint la surface du cours d'eau.

ACKNOWLEDGEMENTS

Claire Kent and her colleagues at Washburn and Gillis Assoc. conducted the field sampling for pesticide residue, and did the hydrological calculations necessary for estimating pesticide residence times.

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1.0 INTRODUCTION

White spruce plantations in New Brunswick are currently infested with epidemic populations of the spruce budmoth (Zieraphera canadensis (E. Kettela, pers. comm.). Attempts to control this insect with presently registered forestry pesticides and conventional spray technology have failed due to the fact that the insect creates its own protective microhabitat. Immediately after hatching the budmoth moves to the apical shoots and webs the bud cap to the tightly closed bud. This not only prevents the shoot from flaring, but also makes it extremely difficult for pesticide droplets to penetrate the tightly closed bud needles while the larvae "mine" the food supply up to pupation. The larvae are most vulnerable when they are migrating from the bud base where eggs are hatched, to the apical shoots where they feed.

Early in 1984, the Forest Pest Management Institute (FPMI) of the Canadian Forestry Service proposed a program to determine the effectiveness of aminocarb (as Matacil 180F) applied at 180 g ai/ha, during the period of larval migration, in controlling this pest. Since this dosage rate is twice the maximum dosage rate registered for spruce budworm (90 g ai/ha), a number of environmental agencies expressed concerns relative to the environmental consequences of such a treatment. Accordingly, the Water Quality Branch, Environmental Conservation Service and the Environmental Protection Service undertook a cooperative investigation to determine the fate and effects of these high dose aminocarb applications on aquatic systems. The Water Quality Branch designed a study to evaluate the concentrations of aminocarb in the stream environment and the Environmental Protection Service designed a complementary study to determine the impacts on biota.

2.0 METHODS

2.1 Site Description

With the assistance of J.D. Irving Ltd. and FPMI personnel, a site was selected in the white spruce plantation areas of J.D. Irving Ltd., which was scheduled to receive the Matacil 180F treatment. The treatment stream selected was Hayes Brook, a small tributary of the Little Main Restigouche River which drains a 125 ha area. Another tributary of the Little Main Restigouche, Jardine Brook, served as a control stream covering approximately 3 times the area of the treatment stream. The original intention was to situate the treatment stream in a relatively large (500 ha) spray block which would have encompassed most of the stream's watershed. FPMI staff later reduced the spray block's size, however, to 100 hectares, of which only a few hectares were in the stream basin. Arrangements were then made to spray a small portion of the study stream directly (Figure 1).

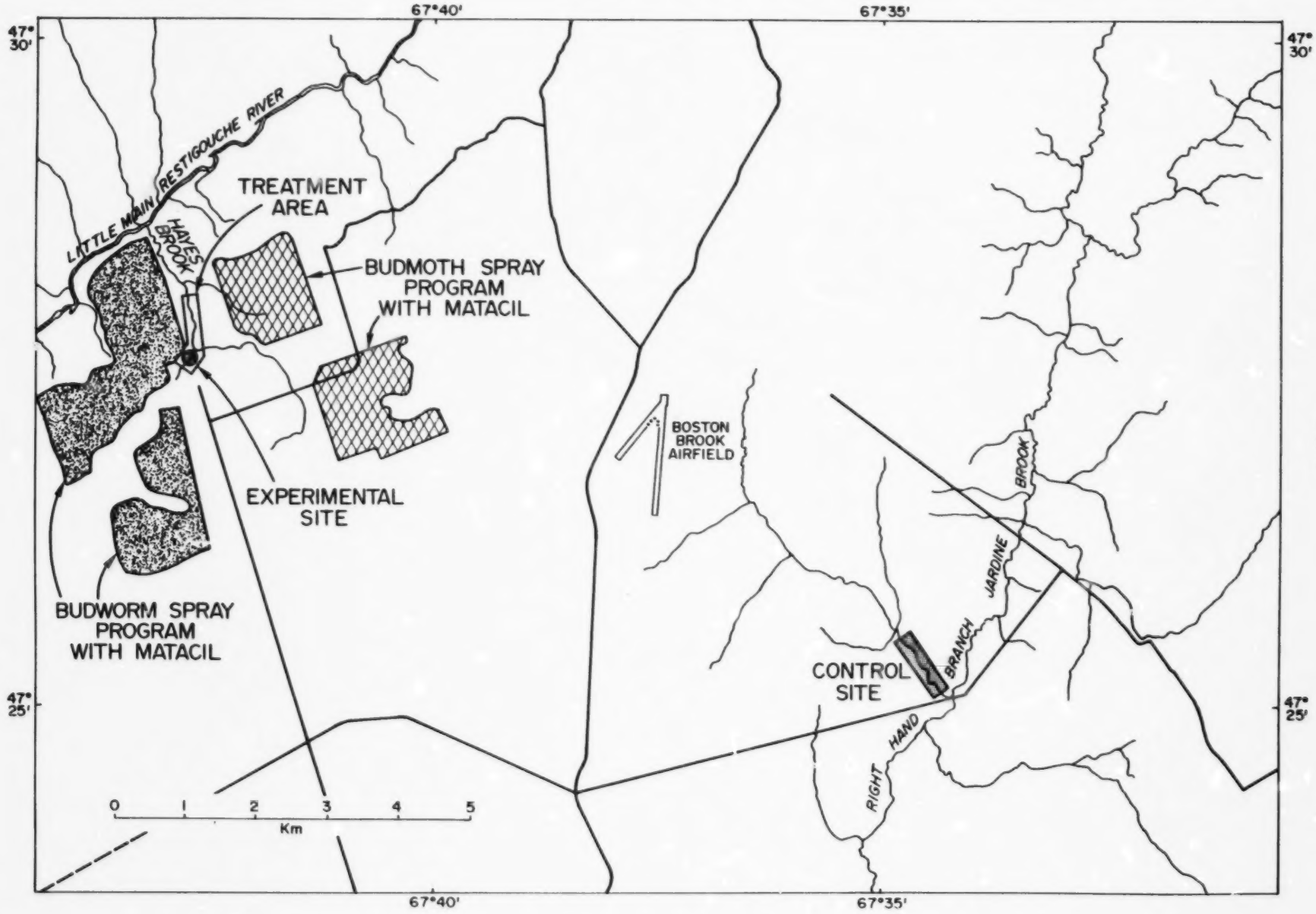
2.2 Experimental Design

Pesticide residue concentrations were determined in water, sediments and caged shellfish (Anadonta Spp.) prior to and at various times after spray. There was also an attempt to obtain samples for residue analyses after significant rainfall events, in order to determine if washoff from foliage and soil runoff would result in additional levels of pesticide in the aquatic system. For the purposes of this study, a significant rainfall event was defined as any event where rainfall was greater than 5.0 mm.

The effects of treatment on aquatic invertebrates was measured by monitoring aquatic invertebrate drift and benthic biomass abundance before and at various times after the spray event. Effects on caged fish (Salvelinus fontinalis) were determined by measuring brain acetylcholinesterase activity before and at various times after the spray event.

FIGURE 1

EXPERIMENTAL SITE LOCATIONS


67°40'
67°35'
47° 30'
47° 25'
LITTLE MAIN RESTIGOUCHE RIVER
HAYES BROOK
TREATMENT AREA
BUDMOTH SPRAY PROGRAM WITH MATACIL
EXPERIMENTAL SITE
BUDWORM SPRAY PROGRAM WITH MATACIL
BOSTON BROOK AIRFIELD
CONTROL SITE
JARDINE BRANCH BROOK
RIGHT HAND
0 1 2 3 4 5
Km

2.3 Treatment

On May 21 at 0845 a single direct spray of Matacil 180F emitted at a dosage rate of 180 g ai/ha, was applied to approximately 300 metres of Hayes Brook + 100 ha of the stream's basin. The application was made with a Thrush Commander aircraft equipped with Micronair spray units. The insecticide was formulated in water and applied at 5 litres of spray mix per hectare.

2.4 Sampling

Water samples were taken in 1 litre amber glass bottles which had previously been detergent washed, and then rinsed with deionized water and hexanes. Water samples were preserved immediately by adjusting the pH to 3.5 with technical grade sulfuric acid. Single water samples were taken prior to spray (25 hours) and at 4, 8, 12 hours after spray. Triplicate samples were taken 24 hours after spray. On May 23-24 approximately 27.2 mm of rain fell in the vicinity and water samples were taken at 12 hour intervals commencing at 12 hours after the onset of the rain event and continuing for a period of 72 hours.

Sediment samples were obtained by scooping available sediment into hexane rinsed foil trays and frozen immediately. Sampling periods were the same as those for water with the exception that samples after the precipitation event were only taken at 48 hours after the onset of precipitation.

The freshwater mussels were collected in Grand Lake, Nova Scotia and placed in wire baskets in each of the study streams three days prior to treatment. At the same time that sediment samples were taken, 10 shellfish were removed from the cage, wrapped in hexane rinsed aluminum foil and immediately frozen.

Residue analyses were conducted by IWD staff in Moncton, N.B. using previously published methods (WQB, 1979).

Artificial substrates (1 litre of approximately 1 cm diameter gravel in bags) were placed in each stream 3 weeks prior to the spray date to allow for adequate aquatic invertebrate colonization. At one day prior to, as well as 24 and 48 hours after the spray event gravel bags were removed from the stream and immediately preserved in 100% isopropanol.

Duplicate drift samples were taken with drift nets (30 cm x 30 cm opening) placed in the tails of riffles. Drift nets were fished for 15 minutes. Water flow was measured through the nets using a General Oceanics Model 2030 digital flowmeter.

One day prior to spray drift samples were taken every 2-4 hours over a 24 hour period in order to establish a diel periodicity. Drift samples were collected ½ hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours after the spray event and immediately preserved in 100% isopropanol.

All aquatic invertebrate samples were subsequently sorted according to the most suitable taxonomic category (generally genus).

Approximately 50 brook trout averaging 15 cm in length were obtained from the Fisheries and Oceans Canada hatchery at Saint John, New Brunswick and held in cages (10 fish per cage) in each stream. Prior to spray and at 2, 12, 24, 48, 72, 84, 96, 340 hours after spray six fish were removed, and heads severed. The latter were wrapped in aluminum foil and frozen immediately. Heads were later thawed, brains removed, weighed and cholinesterase activity determined using the technique of Hill and Flemming (1982) (Appendix 1).

3.0 RESULTS

The characteristics of the control and treated streams are contained in Table 1.

No aminocarb was detected in water or sediment samples from Hayes Brook (treated) collected within the 24 hour post-spray period. After the rainfall event only one water sample had a concentration of aminocarb (0.11 ug/L) above the detection limit (0.001 ug/L). This sample was taken at 0800 on May 25, 36 hours after the rainfall event began. Aminocarb levels in all sediment and mussel samples were below the detection limits.

The number of identified aquatic invertebrates in drift samples taken before and after spray are contained in Tables 2 and 3. The drift samples indicated that the untreated stream (Jardine Brook) had greater populations of aquatic invertebrates than did the treated stream (Hayes Brook). The total number of aquatic invertebrates in the drift of the treatment stream did not change appreciably after the spray.

The benthic invertebrates colonized artificial substrates in the control stream (Table 4) in far greater numbers than in the treatment (Table 5) stream. There was no decrease in the total number of invertebrates in the treated stream which could be related to the pesticide treatment. There was, however, a substantial increase in total numbers of invertebrates two weeks after the spray event and most of this increase was due to larger numbers of Pentaneura Sp in the samples.

The results of brain acetylcholinesterase assays (Table 6) indicated that there was no depression of enzyme activity after spray, compared with pre-spray and control fish. Two fish were found dead in the treatment stream cages at 72 hours and 96 hours after spray and the brain acetylcholinesterase assays on these fish were slightly depressed, however the depression was not appreciable.

TABLE 1 CHARACTERISTICS OF STREAM CONDITIONS AT VARIOUS TIMES DURING THE STUDY

SITE	DATE	TEMPERATURE °C	pH	MEAN VELOCITY (cm/sec)	MEAN FLOW RATE (litres/sec)
Jardine Brook	May 19	5.0	6.8	117	63
(control)	May 21	8.0		58	32
	May 23	16.0		59	32
	May 24 a.m.	10.5	6.6	95	52
	May 24 p.m.	11.5	6.5	116	63
	May 25	7.0	7.0	110	60
Hayes Brook	May 19	3.0	7.2	50	94
(treated)	May 21	5.0		58	109
	May 24 a.m.	6.6	6.6	116	217
	May 24 p.m.	6.0	7.0	86	160
	May 25	4.5	7.0	57	107

TABLE 2 NUMBER OF IDENTIFIED AQUATIC INVERTEBRATES IN STREAM DRIFT OF JARDINE BROOK (UNTREATED*)

ORDER	FAMILY	GENUS	AVERAGE NO. INVERTEBRATES/SAMPLE												
			PRE-SPRAY						POST-SPRAY						
			0730	1000	1145	1500	1720	1910	1/2	2	4	8	24	48	96
Ephemeroptera	Siphlonuridae	Parameletus				1.5							1.5		
		Ameletus		1		3.5									
	Leptophlebiidae	Paraleptophlebia		2		4	1	0.5		2	2.5		0.5		4
		Leptophlebia		0.5	5.5	0.5									
		Chloroterpes			6.5			1							
	Ephemerellidae	Ephemerella		0.5	2	0.5		0.5		1.5					1
	Heptageniidae	Stenveron				0.5				2		0.5			1.5
		Stenonema		3	2.5						1.5		0.5		18
		Epeorus		0.5	6	1		0.5		12	2	0.5		1.5	32
		Heptagenia				0.5				0.5					12
	Baetidae	Pseudocloen		6.5	4		1								
	Ephemeridae	Hexagenia			1										
Trichoptera	Brachycentridae	Brachylentrus			0.5			0.5				0.5			1
		Micrasema													0.5
	Rhyacophilidae	Rhyalophila					0.5							0.5	1
	Lepidostomatidae	Lepidostoma		2		1	1.5	3.5		0.5			0.5		2
	Limnophilidae	Ironoquia		1	1										

Cont'd

TABLE 2 (Cont'd)

ORDER	FAMILY	GENUS	AVERAGE NO. INVERTEBRATES/SAMPLE												
			PRE-SPRAY						POST-SPRAY						
			0730	1000	1145	1500	1720	1910	1/2	2	4	8	24	48	96
Plecoptera	Taeniopterigidae	Taeniokema				1.5		2	0.5	0.5			0.5		1.5
	Chloroperlidae	Hastaperla		0.5											
		Alloperla						0.5					0.5		1
	Perlodidae	Isoperla								0.5					
Diptera	Rhagionidea	Atherix		0.5		0.5	0.5				0.5				25
	Simuliidae	Simulium		1.5	0.5					0.5					
	Heleidae	Atrichopogon											0.5		
Collembola	Isotomidae	Isotoma						1.5					0.5		
		Isotomurus								1.5			2	0.5	3.5
Coleoptera	Elmidae	Narpus		2.5	3			0.5							
		Orodbrevia				1.5									
	Pytiscidae	Agabus	0.5		0.5	1.0									
TOTAL			0.5	20.0	32.5	17.0	4.5	9.0	0.5	21.5	6.5	1.5	8.0	3.5	81.5

* Values presented are means of two 15 minute drift samples.

TABLE 3 NUMBER OF IDENTIFIED AQUATIC INVERTEBRATES IN STREAM DRIFT OF HAYES BROOK (TREATED*)

ORDER	FAMILY	GENUS	AVERAGE NO. INVERTEBRATES/SAMPLE												
			PRE-SPRAY						POST-SPRAY						
			0700	0900	1100	1600	1800	2000	1/2	2	4	8	24	48	96
Ephemeroptera	Siphlonuridae	Parameletus	1				1								
		Ameletus		0.5	0.5	2.5		0.5			1.5				
	Leptophlebiidae	Paraleptophlebia									0.5				
	Heptageniidae	Stendnema						0.5			0.5				
		Epeorus	1		0.5	2		0.5	0.5	0.5	3			3	2
	Baetidae	Pseudocloen			0.5	0.5									
Trichoptera	Brachycentridae	Brachycentrus	0.5				0.5								
		Micrasema													0.5
	Rhyacophilidae	Rhyalophila	0.5												
	Polycentropodinae	Polycentropus		0.5											
	Lepidostomatidae	Lepidostoma	0.5	0.5		0.5		0.5	0.5						
	Hydropsychidae	Macrosema				1									
		Parapsyche													
		Cheamatopsyche									0.5				
	Limnophilidae	Lonqula			1										
	Psyghomeidae	Psychomyia									0.5				
	Philoptamidae	Wormaidia					0.5								

Cont'd

TABLE 3 (Cont'd)

ORDER	FAMILY	GENUS	AVERAGE NO. INVERTEBRATES/SAMPLE												
			PRE-SPRAY						POST-SPRAY						
			0700	0900	1100	1600	1800	2000	1/2	2	4	8	24	48	96
Plecoptera	Taeniopterigidae	Taenionema	0.5				0.5	0.5	1.5	1.5	2.5		0.5	0.5	1.5
	Chloroperlidae	Hastaperla	1.5		0.5	1									
		Alloperla							0.5		0.5				
	Perlodidae	Isoperla								1					
Diptera	Chironomidae	Pentaneura			1						0.5				
	Simuliidae	Simulium		0.5	0.5	1									
	Tabanidae	Crysops												0.5	
	Schizomyidae	Poechographa									0.5				
	Tipulidae	Lipsothrix	0.5												
	Anthomyidae	Limnophora												0.5	
Collembola	Isotomidae	Isotomulus	0.5	0.5	0.5									1	
		Isotoma										0.5			
Coleoptera	Elmidae	Narpus	0.5												
	Limnichidae	Luthrochus				0.5									
	Psephenidae					0.5									
	Limnebiidae					0.5									
TOTAL			7.5	3.5	5	10	2.5	2.5	3.0	3.0	10.5	0.5	0.5	6.5	5

* Values presented are means of two 15 minute drift samples.

TABLE 4 IDENTIFIED INVERTEBRATES IN ARTIFICIAL SUBSTRATES TAKEN FROM JARDINE BROOK (UNTREATED)

TAXA			TIME				
ORDER	FAMILY	GENUS	PRE-SPRAY	1 DAY POST	2 DAYS POST	4 DAYS POST	2 WEEKS POST
Ephemeroptera	Ephemerellidae	Ephemerella	1, 3, 1,	3, 2,1, 5, 1, 2	3, 1, 1	1, 2, 2, 1, 1	1, 2, 2, 1
	Leptophlebiidae	Paraleptophlebia	2, 5, 4, 4, 1, 1	8,10, 4, 3, 1	8, 5, 1	4, 4, 4, 2, 2	1, 5, 8, 7
	Heptagenidae	Epeorus	3, 6, 2	5, 1, 15,13	7, 1, 6, 8	1, 5, 7,10, 5	3, 3, 9, 5, 3, 8
		Stenonema	2, 1	1, 1, 1	2, 1	1, 2	2, 1
		Heptagenia	2	4, 1	2, 2, 1	2, 4	2, 4, 1
	Siphlonuridae	Siphloneurus		1			
		Parameletus			1		1
Trichoptera	Brachycentridae	Brachylentrus	2			1,	1
	Hydropsychidae	Macronema		2			
		Cheumatopsyche		1	1	1	
	Rhyacophilidae	Rhyalophila	1, 1, 3	1			1
	Hydropsychidae	Macronematinae		1	3		1
	Brachycentridae	Micrasema	1				1
Plecoptera	Taeniopterygidae	Taenionema	1, 2	2, 2, 1, 1, 1	4, 1, 2, 4	1, 1, 1, 1	1 2
	Chloroperlidae	Alloperla		1		1, 1	1
		Paraperla					3

Cont'd

TABLE 4 Cont'd

ORDER	TAXA FAMILY	GENUS	TIME PRE-SPRAY	1 DAY POST	2 DAYS POST	4 DAYS POST	2 WEEKS POST
Diptera	Chironomidae	Pentaneura	7,10, 4, 9,10	1, 3, 7, 2	12,12, 4	1,34, 3,10, 7	1, 4, 5, 2, 3, 3,34
	Simuliidae	Simulium	1, 2, 1	1	4, 4	1, 2, 10, 4	1, 2, 13
	Rhagionidea	Atherix	3, 3, 6, 3,2	12, 3, 2, 5	9, 1, 2, 4, 1,13	1, 5, 9,19,14, 7	3, 4,10,11,15, 4
	Tipulidae	Hexatoma			1		1, 1, 1
	Heleidae	Probezia			1		
	Psychopidae						2
Collembola							
Coleoptera	Elmidae	Narpus Rhizelmis	1, 5,10, 3, 5 1	2, 3, 14, 5, 5	10,11,12, 10,10 3	7, 6,20,19, 2,11	22,36,8,22,21,13
Oligochaeta					1		1
"Hydracarina"					1	3	2
TOTAL			23,20,25,35,26,6	40,18,1,31,36,33	57,27,37,23,12,35	19,50,49,73,45,27	34,52,46,54,50,47,46

TABLE 5 IDENTIFIED INVERTEBRATES IN ARTIFICIAL SUBSTRATES TAKEN FROM HAYES BROOK (TREATED)

TAXA			TIME				
ORDER	FAMILY	GENUS	PRE-SPRAY	1 DAY POST	2 DAYS POST	4 DAYS POST	2 WEEKS POST
Ephemeroptera	Ephemerellidae	Ephemerella	1	1			
	Heptagenidae	Epeorus		1, 6	1	3, 1	1
		Stenonema		1, 8			
	Leptophlebiidae	Paraleptophlebia		3			
Trichoptera	Polycentropodidae	Polycentropus	1	1,1	3	1	2, 1
	Rhyacophilidae	Rhyalophila		1,1	2	1,1, 3	
	Limnephilidae	Ironoquia	1				
	Brachycentridae	Brachycentrus		1	1 1	1	
		Micrasema			1		
	Odontoceridae	Psilotreta				1	
	Hydropsychidae	Arctopsyche					1
	Polycentropidae	Nyctio					
	Psychomyiinae	Lype				1	
Plecoptera	Chloroperlidae	Alloperla	1,2	2, 2,1	3, 17,1,2	1, 1	12, 4
	Taeniopterygidae	Taenionema	5,5	1,1, 1	1, 4	8,1,1,2	5,12
	Chloroperlidae	Hastaperla		1		4	1
		Chloroperla			1		

Cont'd

TABLE 5 Cont'd

TAXA			TIME				
ORDER	FAMILY	GENUS	PRE-SPRAY	1 DAY POST	2 DAYS POST	4 DAYS POST	2 WEEKS POST
Diptera	Rhagionidea	Atherix	1	4	3	1, 1 1	2, 2
	Chironomidae	Pentaneura	1	5, 2 4	4, 8, 2	6, 6, 9,10	38,46,50,29,41
	Simulidae	Simulium		2		1	2
	Schizomidae	Poecilographa			1		2
	Tipulidae	Hexatoma Lipsothrix				1	
Collembola							
Coleoptera	Elmidae	Narpus		1 2			
"Hydracarina"				1			
Oligochaeta						1, 1, 1	1, 1
TOTAL			6,2,5,5	8,4,1,6,4,32	10,15,17,8,5,1	8,5,15,8,15,19	59,58,61,30,45

TABLE 6 BRAIN ACETYLCHOLINESTERASE ACTIVITY OF FISH IN CONTROL AND TREATED STREAMS BEFORE AND AT VARIOUS TIMES AFTER SPRAY

TIME	BRAIN ACHE ACTIVITY (uM HYDROLYZED/g/min x 10^4)	
	JARDINE BROOK (UNTREATED)	HAYES BROOK (TREATED)
Prespray	5.0	8.4
Prespray	6.7	
Prespray	4.5	
2 hr Post Spray	6.1	
2 hr Post Spray	6.3	8.1
2 hr Post Spray	5.2	9.5
12 hr Post Spray	7.3	10.0
12 hr Post Spray	6.5	8.5
24 hr Post Spray	7.7	7.2
24 hr Post Spray	8.5	7.4
48 hr Post Spray	6.2	7.8
48 hr Post Spray	6.8	5.8
72 hr Post Spray	7.1	7.9
72 hr Post Spray	6.5	7.8
84 hr Post Spray	6.8	8.1
84 hr Post Spray	6.5	9.8
2 weeks Post Spray	6.2	7.6
2 weeks Post Spray	6.6	6.8
Dead Fish		
72 hr Post Spray		4.5
72 hr Post Spray		4.3
72 hr Post Spray		4.8
96 hr Post Spray		5.9
96 hr Post Spray		5.4

4.0 DISCUSSION

It has been demonstrated that stream drift benthic invertebrate abundance (Eidt, 1975) and fish acetylcholinesterase activity (Jarvinen et al., 1983) are sensitive indicators of aquatic pesticide contamination. The combined results of stream invertebrate drift, benthic invertebrate abundance and fish brain acetylcholinesterase assays indicate that there was little if any impact on the treatment stream from the pesticide application. The death of two fish (approximately 5% of the exposed population) in the treatment stream after the pesticide application cannot be conclusively attributed to pesticide poisoning. Due to the lack of evidence of pesticide impacts on aquatic invertebrates, a more likely cause of the fish deaths is physical damage caused by increased water flow rates through the holding cages.

While operational aminocarb treatment at spruce budworm dosage rates of two applications of 70 g ai/ha have not been generally observed to cause major aquatic perturbations (NRCC 1982), the threshold for aquatic impacts is probably close to this dosage rate (Ahern, 1980). It was anticipated, on this basis, that there would have been measurable aquatic impacts with the dosage rate (180 g ai/ha) used in this study. It must be presumed, therefore, that stream deposit of pesticide was not sufficient to produce measurable effects on biota. This would seem to be corroborated by the residue data.

The drainage area of Hayes Brook is approximately 125 ha. The width of the spray swath was estimated to be 60 m and the length of the spray line over the brook was estimated to be 350 m. At a dosage rate of 180 g/ai/ha, a total of 380 g of active ingredient was applied over the stream.

It has been estimated that between 10 and 23 percent of pesticide emitted during an aerial spray will be deposited on the ground (Basak and Picot, 1984). Using this relationship, we can assume that between 38 g and 87 g of aminocarb reached the sprayed portion of the stream. Hayes Brook is, on average, 0.72 m wide and 0.26 m deep and we sampled approximately 200 m downstream from the beginning of the spray swath, so the volume of water in the sprayed area above the sample site would be approximately 37.4 m^3. When the aminocarb was applied, the stream discharge was 0.058 m^3/s, and the first water sample was taken four hours after the spray application. It is not surprising then, that aminocarb was not detected in water after the spray since by this time the stream had been flushed approximately 22 times and it is highly probable that the amount of aminocarb available had been washed through the experimental section of Hayes Brook.

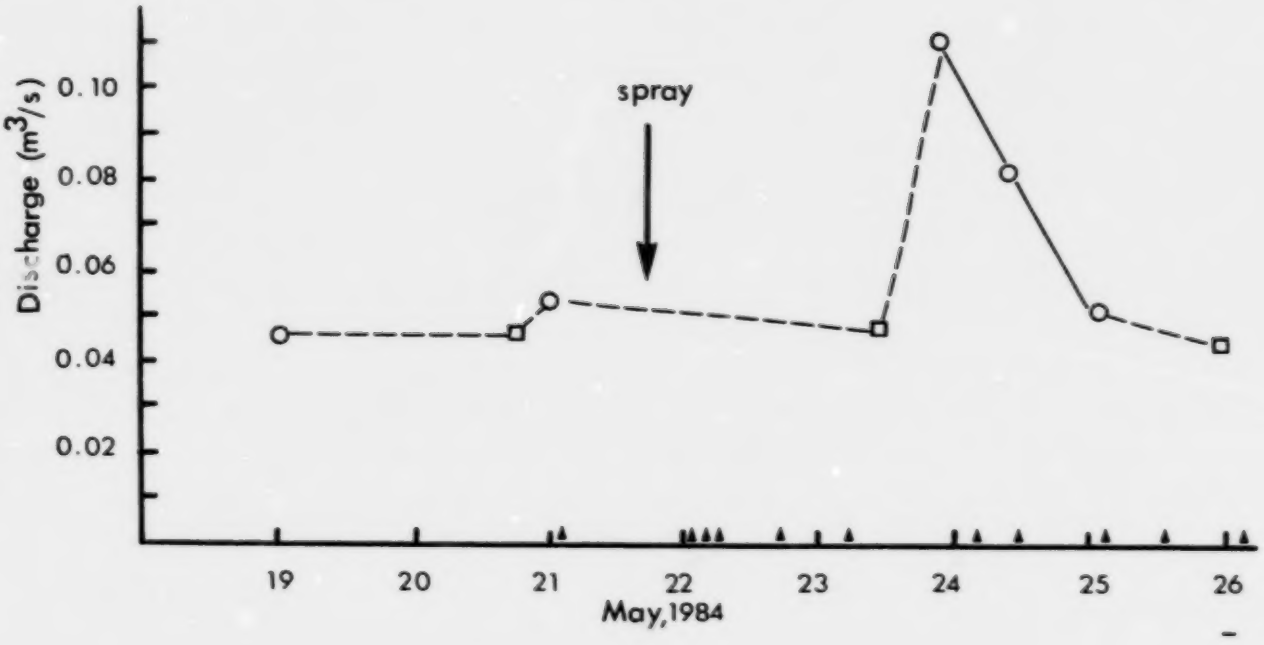
To measure levels of aminocarb after a significant rainfall event, it was presumed that a large part of the drainage basin of the sampling site would be sprayed, when in fact, a very small portion of the stream basin was actually sprayed.

To attempt to assess why the only sample which had a reading above the detection limit was the sample taken one and a half days after the onset of rain, it was necessary to estimate when the peak flow occurred. If this sample was taken before or close to peak flow, then we could assume that the occurrence of measurable aminocarb in only this sample might be expected.

Measured stream flow values were used to plot the discharge hydrograph shown in Figure 2. The dashed portions of the hydrograph are assumed based on the rainfall events at Veneer located approximately 15 km from the spray site. Also shown are the times when the spray was applied and when water samples were taken.

FIGURE 2

APPROXIMATE DISCHARGE HYDROGRAPH FOR HAYES BROOK



Legend: ○ recorded discharge
□ assumed discharge based on rainfall records
▲ water samples taken

It appears that the spray was applied at a time of relatively low stream flow. The rainfall event of 23-24 May, caused the flow to approximately double and after the rainfall, the stream discharge decreased rapidly. When the sample was taken, the discharge rate had returned to the same level as when the spray was applied.

The total runoff volume from the event of 23-24 May was estimated to be 4620 m^3 from the entire watershed. The rational method[1] (Linsley and Franzini, 1979) was used to estimate the time required for a particle of water to travel by overland flow from the most remote part of the watershed to the outlet (time of concentration). Although the results of this method are only approximate, the time was estimated to be from 10 minutes for water precipitated near the sampling site to 12 hours for water originating at the furthest part of the watershed. If so, then this mixture from the sprayed portion would enter the channel very soon after the rainfall began. Therefore, there is a strong possibility that runoff during the early hours of the rainfall event of 23-24 May, may have contained aminocarb. Samples taken after 12 hours past the onset of rain would be most unlikely to contain the sprayed pesticide.

As aminocarb was not detected in two samples taken at higher flows on 24 May, 12 hours and 24 hours after the onset of rain, it is most likely that the measured value was due to contamination unless some unaccounted effects such as delayed solubility of aminocarb or the sudden release of surface storage (for example, a puddle overflowing).

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1 The rational method is given by $Q = iA$, when Q is the peak rate of runoff, i is the intensity of rainfall for duration = time of concentration, and A is the watershed area.

This study then shows that any potential impacts on the biota would have been restricted to two very short periods of approximately one hour maximum. One period of exposure was during the actual overspray, and the other within a few minutes of the onset of the rainstorm. Considering the concentrations of aminocarb involved, and considering its toxicity to aquatic life, it is unlikely that the stream overspray would cause any measureable impacts on the biota.

5.0 RECOMMENDATIONS

In a stream as small as Hayes Brook, four hours after a direct over-spray is likely too long a time lapse to pick up peak concentrations of a pesticide. As shown in previous calculations, in four hours the stream would have been flushed approximately twenty-two times. Samples should be taken as soon after the spray as possible (within 15 minutes), with as much of the stream sprayed as possible.

In future sampling, where levels of a pesticide are to be measured after a rainfall event, it is recommended that a sampling procedure be designed for the specific site, with the procedure taking into account how quickly a stream would respond to a rainfall event. To estimate this roughly, the size of the watershed, the slope of the drainage basin and the type of vegetation in the basin would have to be considered in advance. This estimate would indicate approximately how often water samples and stream flow measurements should be taken. It should also be noted that the stream response time would depend on the intensity of the rainfall. Measurements such as stream flow, cross sectional area and accurate rainfall data (from a rain gauge) would give a better idea of when we would expect to find higher concentrations of pesticide.

Ideally, on a stream the size of Hayes Brook, flow measurements and water samples should be taken hourly during a rainfall event until the flow peaks and returns to pre-rain levels. In Hayes Brook the time to return to pre-rain levels after the rain event of 23 May was approximately 36 hours.

Considering that the more important part of the hydrograph, is the rising limb, samples should be taken hourly until the discharge peaks, then at less frequent intervals: 2, 4, 6, 12, 18, 24 hours. Analyses could be performed on every fourth to sixth sample with more

samples analyzed if necessary. All samples should be taken in duplicate, but all need not be analyzed unless an aberrant feature as was seen in the sample taken at 0800 hr. on the 25 May occurred.

Finally, the water concentrations of pesticide and associated impacts on biota will be maximal, and more closely approximate operational spraying, if the entire watershed is sprayed. For this reason, total watershed experimental treatments should be preferred over limited "stream only" treatments.

6.0 REFERENCES

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APPENDIX I

ACETYLCHOLINESTERASE ACTIVITY DETERMINATION

Three reagents are required for the assays.

A. Chromagin Reagent:

DTNB (dithiobisnitrobenzine)	9.9 mg
Trizma HCl	661.0 mg
Trizma base	97.0 mg

Made up to 100 ml with deionized water refrigerated and stored in a dark bottle (shelf-life 1-2 months).

B. Substrate:

Acetylthiocholine Iodide	451.1 mg

Made up to 10 ml with deionized water refrigerated and stored in dark bottle (shelf-life 2-4 days).

C. Tris Buffer:

Trizma HCl	4.44 g
Trizma base	2.65 g

Made up to 10 ml with deionized water and stored at room temperature (shelf-life indefinite).

Whole brains are weighed and Tris buffer (reagent C) added in the proportion of one part brain to ten parts of reagent by weight and homogenized using a micro homogenizer, for 60 seconds. It is important that duration of homogenization be consistent from sample to sample.

To a spectrophotometric cuvette (1 cm optical path) add 2.9 ml of Chromagen Reagent (Reagent A) and allow it to warm to room temperature. Next add 20 uL of the homogenate and 100 ul of substrate to the cuvette, mix well with a

plastic stirring rod and allow to stand for 45 seconds. Absorbance is then measured on a spectrophotometer (e.g. Coleman Model 46) operating at a wavelength of 405 nm. The reaction is allowed to progress for 60 seconds and the absorbance is measured again. The enzyme activity is calculated by multiplying the change in absorbance over 1 minute by 1.22×10^6 moles to provide the micromoles of substrate hydrolyzed per minute per gram of tissue.